REMARKS

I. Support for the Amendments

Claims 1-26 were originally in the application. Non-elected claims 8-26 were previously withdrawn. Claims 2 and 4-7 were canceled during previous Amendments.

Claims 1, 3, and 27-36 were the subject of the present Office Action. Claim 1 has been allowed. Claims 27 and 32-35 are amended, previously withdrawn claims 8-26 are canceled without prejudice, and new claims 37-57 are added, as shown in the following listing of claims, which will replace all prior versions and listings of claims in the application. Claims 8-26 were previously withdrawn from consideration as the result of a restriction requirement and are hereby canceled without prejudice to their pursuit in an appropriate continuation or divisional application.

Support for amended claims 27 and 32-35 and for new claims 37-57 can be found in the original specification, figures, and claims. In part, the amendments to claims 33 and 34 change their dependency. In part, the amendments to claims 32-34 make their language more consistent with the language of claim 27. Additional support for amended claims 27 and 35 and for new claims 37, 41, 43, 47, 48, 52, 53, and 57 can be found, e.g., on pages 7-8 and 14; in Figures 2, 3, and 13-15; and in the Examples, especially Examples 2 and 6. Additional support for amended claim 32 and for new claims 40-43, 48, and 53 can be found, e.g., on pages 7-9, 11-16, and 18-19; in the Figures; and in the Examples. Additional support for new claims 37-57 can be found, e.g., on pages 6-7, 10-11 and 17-18; in the Figures (e.g., Figures 6 and 7); and in the Examples. Additional support for new claims 38, 39, 41, 43-46, 48-51, and 53-56 can be found, e.g., on pages 7-19 (e.g., pages 3, 10-12, and 17-18); in the Figures; and in the Examples. Additional

support for new claim 41 can be found, e.g., on pages 8-9, 12-13, and 15-16; in the Figures (e.g., Figures 2, 3, 11, and 14); and in the Examples.

II. Status of the Claims

Claims 1-26 were originally in the application. Claims 1-26 were subject to an election/restriction requirement, and claims 1-7 were elected. Claims 8-26 were withdrawn without prejudice or disclaimer of any subject matter. Claims 2 and 4-7 were previously canceled, and new claims 27-36 were previously added.

Claims 1, 3, and 27-36 were the subject of the present Office Action. Claim 1 has been allowed. Claims 27 and 32-35 are amended, previously withdrawn claims 8-26 are canceled without prejudice, and new claims 37-57 are added, as shown in the following listing of claims, which will replace all prior versions and listings of claims in the application. Claims 8-26 are hereby canceled without prejudice to their pursuit in an appropriate continuation or divisional application.

III. The Drawings and the Certified Copies of the Priority Applications

Applicants thank the Examiner for accepting the drawings and for acknowledging receipt of the certified copies of all priority applications.

IV. Rejection of Claims 3 and 27-36 under 35 U.S.C. §112, First Paragraph is Traversed

The Examiner has entered the Amendment mailed has rejected claims 3 and 27-36 under 35 U.S.C. §112, first paragraph, for reasons relating to enablement. Applicants thank the Examiner for entering the Amendment, but respectfully traverse the rejection.

The Office Action states:

> As stated in the previous Office Action the standard for meeting the enablement requirement is whether one of skill in the art can make the invention without undue experimentation. However, the limitations that the claimed nicotianamine synthase variants must have activity of more than 25% of the nicotianamine synthase activity of SEQ ID NO: 1 and that the activity is measured by an assay does not enable one of skill in the art to make the invention without undue experimentation: These limitations require searching and screening for the invention which is not teaching or guidance for making the invention
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> The amount of experimentation to make the nicotianamine synthase having 50%, 90%, and 95% identity to SEQ ID NO: 1 comprising at least one of amino acid sequences SEQ ID Nos: 23-28 recited in claim 27 and having 101 conserved amino acids recited in claim 28 is undue for the reasons stated in the previous Office Action. SEQ ID NO: 1 is disclosed by the specification as an amino acid sequence of 328 amino acid residues. The claims require at least 50% of SEQ ID NO: 1 to be altered, where at least 164 amino acid residues are changed (deletion, insertion, substitution, or combinations thereof) in SEQ ID NO: 1 and 101 amino acids must be conserved. One of ordinary skill in the art would have to make and search for proteins having these changes in the amino acid sequence and then determine by enzymatic assays whether the protein has nicotianamine synthase activity. Limiting the claims to recite the specific amino acid sequences of SEQ ID Nos: 23-28 does not overcome the rejection since no more than 32 amino acid residues are accounted for.
>
> Furthermore, claims 29 and 30 which recites 90% and 95% identity, respectively, to SEQ ID NO: 1 requires making and screening for 33 amino acid residues that can be altered without inactivating enzyme activity. Thus, one of ordinary skill in the art would have to make and search for proteins having these changes in the amino acid sequence and then determine by enzymatic assays whether the protein has nicotianamine synthase activity. Such making and searching is outside the scope of

routine experimentation.

> The amount of experimentation to make the nicotianamine synthase having 50%, 90%, or 95% identity to SEQ ID NO: 1 comprising at least one of amino acid sequences SEQ ID Nos: 23-28 recited in claim 35 and having 101 conserved amino acids recited in claim 36 is undue. The claims require at least 50%, of SEQ ID NO: 1 to be altered, where at least 33 amino acid residues are changed (deletion, insertion, substitution, or combinations thereof) in SEQ ID NO: 1 and 101 amino acids must be conserved. One of ordinary skill in the art would have to make and search for proteins having these changes in the amino acid sequence and then determine by enzymatic assays whether the protein has nicotianamine synthase activity. Limiting the claims to recite the specific amino acid sequences of SEQ ID Nos: 23-28 does not overcome the rejection since no more than 32 amino acid residues out of a total of 328 amino acid residues of SEQ ID NO:.1 are accounted for. [Pp. 3-4, par. 4.]

Applicants respectfully disagree. Claim 3, as amended, is dependent on new claim 27. As noted previously, the claim language of claim 27, similar to the previous language of claim 1, **does <u>not *require*</u>** 50% of the residues to be non-identical, rather it is simply drawn to "a polypeptide having more than 50% identity," which could include, for example, polypeptides of 51% identity, 65% identity, 90% identity, and 99% identity. Moreover, claim 27 requires the polypeptide to have a nicotianamine synthase activity of more than 25% of an equivalent amount of the nicotianamine synthase activity of the enzyme of SEQ ID NO:1. In essence, there is not only a sequence identity requirement, but also an activity requirement.

While it has been alleged that this language would require undue experimentation, this argument seems to be related primarily to artificial derivatives of nicotianamine synthase, which have been obtained by mutation, such as site-directed mutagenesis. These days, if an isolated DNA encoding an enzyme derived from one specimen and a suitable expression system which can produce the functional enzyme are provided, barring unusual factors, it is not necessarily undue experimentation for one of skill in the art to isolate a DNA encoding a homologous protein from another species or another family member from the same species by using standard genetic engineering techniques, such as PCR or hybridization, and then producing the

homologous protein by using the isolated DNA in the expression system. An appropriate enzyme activity assay can then be used to confirm the identification of the expressed protein. In those situations where the sequence is isolated and/or purified from a natural source, one of skill in the art need not be taught in advance which amino acid residue(s) can be changed without inactivating enzyme activity.

Moreover, Applicants respectfully submit that they have already obtained a number of sequences and that the conserved sequences and individual residues are supported by Figure 7. The Declaration of Dr. Satoshi Mori, a co-inventor of the present application, is submitted herewith. Applicants respectfully submit as one example of enablement, their disclosure of the isolation of OsNAS1 from rice using fragments of SEQ ID NO: 1. The sequence of OsNAS1 is only 75% identical to SEQ ID NO: 1, but appears to have a comparable relative activity (p. 14; Fig. 15).

Reference Figure A, which is discussed in the Declaration and which was submitted with the Amendment mailed on April 15, 2004, shows an amino acid alignment of nicotianamine synthases and identity (%) of included sequences thereof compared with HvNAS1 (SEQ ID NO:1; 100%). Applicants wish to note that the percentages of sequence identity for other barley sequences (SEQ ID NO: 3, 5, 7, 9, and 11) are 61% to 73%, while that of one non-barley sequence of the present invention (rice OsNAS1, SEQ ID NO: 15) is 75%. The figure also shows two additional rice sequences, two additional barley sequences, and three corn sequences. The Examiner's attention is directed to the remarks of Dr. Mori in his Declaration, particularly with respect to the conserved sequences and to the sequences have conservative changes.

Claims 27 and 35 include an assay for measuring activity. As was demonstrated by the present application with respect to OsNAS1, it is possible that other sequences may be less than

90-95% identical (75% for OsNAS1), but still have activity.

In subsequent research this finding has been confirmed. The HvNAS2, HvNAS3, HvNAS4, HvNAS6, and HvNAS7 fusion proteins have nicotianamine synthase activity, despite the fact that the HvNAS2-HvNAS7 sequences have between 61% and 74% identity to SEQ ID NO: 1. The OsNAS2 and OsNAS3 fusion proteins also have nicotianamine synthase activity. The AtNAS1, AtNAS2, AtNAS3, and AtNAS4 fusion proteins have nicotianamine synthase activity, despite the fact that the AtNAS1, AtNAS2, AtNAS3, and AtNAS4 sequences have 47.5%, 44.1%, 48.5%, and 49.0% identity to SEQ ID NO: 1, respectively. The corn sequences, ZmNAS1, ZmNAS2, and ZmNAS3, are 71%, 72%, and 66% identical to SEQ ID NO: 1. ZmNAS2 has additional sequence (insertion) not found in the other enzyme sequences shown. ZmNAS1 and ZmNAS3 have nicotianamine synthase activity, but ZmNAS2 does not.

Applicants also wish to note that this particular application was primarily focused on a protein, which, in an endogenous setting, is found, not in all organisms, but in certain types of plants, and which has an activity that is upregulated in an iron-deficient environment. Limiting the claims to enzymes isolated or purified from plants, however, would not cover artificial sequences. Applicants respectfully request the Examiner to reconsider these points.

Moreover, claim 27 recites a polypeptide having more than 50% identity with SEQ ID NO: 1 and one of six consensus sequences (see Figure 7), in addition to having more than 25% of the nicotianamine synthase activity of an equivalent amount of the nicotianamine synthase activity of the enzyme of SEQ ID NO:1. Similarly, claim 35 recites a mutated polypeptide having more than 95% identity with SEQ ID NO: 1 and one of six consensus sequences (see Figure 7), in addition to having more than 25% of the nicotianamine synthase activity of an equivalent amount of the nicotianamine synthase activity of the enzyme of SEQ ID NO:1.

Figure 7 shows predicted amino acid sequences from seven cDNAs isolated from barley with the conserved amino acid sequences listed below. Therefore, one of ordinary skill in the art would recognize the potential importance of these conserved amino acids, relative to the variable amino acids, with respect to the claimed nicotianamine synthase. In addition, many of the non-conserved amino acid sequences have conservative changes. As Dr. Mori points out in his Declaration, the sequences in Reference Figure A, which include the seven barley sequences and the rice sequences disclosed in the present application, all have at least one of these conserved sequences.

Moreover, the language of claims 27 and 35 also provides that the sequence must have more than 25% of the nicotianamine synthase activity of an equivalent amount of the nicotianamine synthase activity of the enzyme of SEQ ID NO:1. (See, e.g., page 7, second full paragraph; page 14, second full paragraph; page 15, second full paragraph; and the Examples, particularly in Examples 2 and 3.) The language of claims 27 and 35, therefore, provides both sequence parameters and activity parameters. (See, e.g., *Regents of the University of California v. Eli Lilly & Co.*, 119 F.3d 1559, 43 U.S.P.Q.2d 1398 (Fed. Cir. 1997).) As a result, use of part or all of the consensus sequence(s) in the present invention would not require undue experimentation on the part of one of ordinary skill in the pertinent art.

The Examiner notes that some alterations of the sequence may inactivate the enzyme, but there is no requirement that all the embodiments covered by a claim must work. For example, and as the specification makes clear, practice of the invention is not limited to any particular sequence (e.g., pp. 13-19 and the Examples [disclosing particular invention methods in which suitable enzyme fragments or derivatives are used]).

Specific examples of such acceptable sequences are disclosed in the present application, namely, those that have more than 25% relative nicotianamine synthase activity compared to that of HvNAS1. Not only is an example of an assay provided, but the isolation, sequencing, and testing of OsNAS1 are described.

Additionally certain consensus sequences are discussed. Moreover, individual conserved residues are shown in Fig. 7 for example (see also p. 10).

As understood, the rejection takes the position that notwithstanding Applicants' disclosure of specific nicotianamine synthases suitable for use with the claimed invention, in addition to methods for obtaining additional nicotianamine synthases, use of anything but the native enzyme is not enabled on grounds that it would require undue experimentation to make and use the nicotianamine synthases. Applicants respectfully disagree. Moreover, as noted in Dr. Mori's Declaration, Applicants themselves have shown in the application itself an example of just such an isolation and testing, in addition to isolating subsequent sequences.

The specification provides examples of suitable nicotianamine synthases for use with the claimed invention including, but not limited to, the native enzyme. Should use of a particular enzyme fragment or derivative be needed in a specific invention embodiment, the specification provides more than ample guidance about selecting an appropriate fragment or derivative.

For example, a preferred nicotianamine synthase exhibits good activity in the activity assay using ^{14}C-S-adenosylmethionine (SAM) and nicotianamine synthase peptide (p. 14; Examples 2 and 6, pp. 19-20 and 23-25; Figs. 1 and 15).

Moreover, the chemical structure of the barley nicotianamine synthases and a rice

nicotianamine synthase have been disclosed both at the amino acid and nucleic acid levels and the similarity of their amino acid sequences has been compared (e.g., Fig. 7). Consensus sequences have been recognized (p. 10; Fig. 7). Methods for isolating (or mutating) and testing suitable nicotianamine synthases have been disclosed (pp. 13-19; the Examples; the Figures). As discussed by Dr. Mori in his Declaration, these consensus sequences have been observed in OsNAS1, which is also the subject of the present application, and in subsequent work, not only in barley and rice, but also in corn. The relative activity of the OsNAS1 enzyme is comparable to that of the HvNAS1 enzyme encoded by SEQ ID NO: 1.

Accordingly, it is believed that any testing needed to identify or confirm suitable nicotianamine synthases for use with the claimed invention is well within the level of experimentation permitted by the Federal Circuit. *In re Wands*, 8 USPQ2d 1400 (Fed. Cir. 1988).

Applicants disagree with the rejection on other grounds.

For example, a worker in this field would be able to use the guidance provided by the instant disclosure to isolate (or mutate) and test appropriate nicotianamine synthases, such as through the use of convention techniques. Any inoperable embodiments of the type described by the rejection would be demonstrated by the use of an activity assay to test isolated or mutated polypeptides. As described by the Court of Customs and Appeals:

> [M]any patented claims read on vast numbers of inoperative embodiments in the trivial sense that they can and do omit 'factors which must be presumed to be within the level of ordinary skill in the art.' ... There is nothing wrong with this so long as it would be obvious to one of skill in the art how to include these factors in such manner as to make the embodiment operative rather than inoperative. *In re Cook and Merigold*, 169 USPQ 298, 302 (C.C.P.A. 1971) (quoting *In re Skrivan*, 166 USPQ 85, 88 (C.C.P.A. 1970)).

Thus, one of skill having read Applicants' disclosure would know to identify suitable

nicotianamine synthases in addition to the HvNAS1 enzyme encoded by SEQ ID NO: 1. Even if one assumes, *arguendo*, that a particular nicotianamine synthase sequence, fragment or derivative did not exhibit acceptable activity, that result, by itself, would not support the present enablement rejection. The worker would understand that another sequence, fragment or derivative as provided by the specification, could be isolated (or mutated) and then tested and identified for suitable activity. The rejection has not provided any reason to doubt that the guidance provided by Applicants' disclosure could not be used to identify a range of acceptable nicotianamine synthases for use with the claimed invention.

It is noted that the rejection seems premised on the position that only claims drawn to SEQ ID NO: 1 satisfy the requirements of Section 112, first paragraph, notwithstanding the broader invention Applicants disclose (e.g., the OsNAS1 sequence and activity).

Respectfully, such a position conflicts with established patent law. It is well-recognized that a patentee's invention is properly broader than specific embodiments identified in an application. Thus in *In re Anderson*, the CCPA reversed a rejection under Section 112, first paragraph and noted in particular (176 USPQ 331, 333 (C.C.P.A. 1973)):

> What the Patent Office is here apparently attempting is to limit all claims to the specific examples, notwithstanding the clear disclosure of a broader invention. **This it may not do**.... There is no doubt that a patentee's invention may be broader than the particular embodiment shown in his specification. A patentee is not only entitled to narrow claims directed to the preferred embodiment, but also to broad claims which define the invention without a reference to specific instrumentalities. (emphasis added).

Here, the claimed invention is broader than use of the enzyme encoded by SEQ ID NO: 1, as singled-out in the rejection. As taught throughout Applicants' disclosure, the invention is compatible with a variety of suitable nicotianamine synthases including derivatives thereof and those isolated or purified not only from barley, but also from other plant species (e.g., rice) as

well.

In view of the guidance provided by the specification and applicable patent law, the limitation of the claims solely to SEQ ID NO: 1 is simply not needed to practice the invention as claimed.

For the reasons outlined above, Applicants respectfully submit that claims 3 and 27-36 fulfill the enablement requirements of 35 U.S.C: §112, first paragraph. Therefore, Applicants request reconsideration and withdrawal of the rejections made under 35 U.S.C. §112,.first paragraph.

V. The Allowance of Claim 1

Applicants thank the Examiner for allowing claim 1.

CONCLUSION

In view of the foregoing amendments and remarks, the present application is respectfully considered in condition for allowance. An early reconsideration and notice of allowance are earnestly solicited.

It is believed that all outstanding rejections have been addressed by this submission and that all the claims are in condition for allowance. If discussion of any amendment or remark made herein would advance this important case to allowance, the Examiner is invited to call the undersigned as soon as convenient.

Applicants hereby request a three-month extension of time for the Amendment and accompanying materials. If, however, a petition for an additional extension of time is required, then the Examiner is requested to treat this as a conditional petition for an extension of time and the Commissioner is hereby authorized to charge our deposit account no. 04-1105 for the appropriate fee. Although it is not believed that any additional fee (in addition to the fee concurrently submitted) is required to consider this submission, the Commissioner is hereby authorized to charge our deposit account no. 04-1105 should any fee be deemed necessary.

Respectfully submitted,

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